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Insulin Glargine Injection

DEFINITION

Insulin Glargine Injection is a sterile solution of Insulin Glargine in Water for Injection. It has a potency of NLT 95.0 and NMT 105.0 USP Insulin Glargine Units/mL.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solutions*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Buffer: Dissolve 20.7 g of anhydrous monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 2.5, and dilute with water to a final volume of 1000 mL.

Solution A: Dissolve 18.4 g of sodium chloride in 250 mL of *Buffer*, add 250 mL of acetonitrile, and mix. Dilute the solution with water to a final volume of 1000 mL.

Solution B: Dissolve 3.2 g of sodium chloride in 250 mL of *Buffer*, add 650 mL of acetonitrile, and mix. Dilute the solution with water to a final volume of 1000 mL.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	96	4
20	83	17
30	63	37
40	96	4

[Note—Adjust the *Mobile phase* composition and the gradient by a parallel shift to obtain a retention time of 18–23 min for the insulin glargine main peak.]

System suitability solution: Dissolve the contents of 1 vial of USP Insulin Glargine for Peak Identification RS in 0.3 mL of 0.01 N hydrochloric acid, and add 1.7 mL of water.

Standard solution 1: Dissolve the contents of 1 vial of USP Insulin Glargine RS in 1.5 mL of 0.01 N hydrochloric acid, transfer the solution to a 5-mL volumetric flask, and dilute with water to volume. Dilute 4 mL of this solution with water to 10 mL in a volumetric flask.

Standard solution 2: Dissolve the contents of 1 vial of USP Insulin Glargine RS in 1.5 mL of 0.01 N hydrochloric acid, transfer the solution to a 10-mL volumetric flask, and dilute with water to volume.

Standard solution 3: Dissolve the contents of 1 vial of USP Insulin Glargine RS in 1.5 mL of 0.01 N hydrochloric acid, transfer the solution to a 5-mL volumetric flask, and dilute with water to volume. Dilute 3 mL of this solution with water to 5 mL in a volumetric flask.

Sample solution: Quantitatively dilute a portion of Injection with water to obtain a solution containing about 40 USP Insulin Glargine Units/mL.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 3.0-mm × 25.0-cm; 4-µm packing L1

Column temperature: 35°

Flow rate: 0.55 mL/min

Injection volume: 5 µL

System suitability

Samples: *System suitability solution*, *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*

Suitability requirements

Resolution: NLT 2.0 for the ratio of the height of the 0^A-Arg-insulin glargine peak to the height of the valley between the 0^A-Arg-insulin glargine peak and the insulin glargine peak, *System suitability solution*

Tailing factor: NMT 1.8 for the insulin glargine peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, calculated from six response factors from two duplicate injections each of *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*

Analysis

Samples: *Standard solutions* and *Sample solution*

Measure the responses of the major peaks. Prepare a calibration curve based on the peak responses from the *Standard solutions* versus the concentrations (USP Insulin Glargine Units/mL) using linear regression.

Calculate the potency, in USP Insulin Glargine Units/mL, of the portion of Injection taken:

$$\text{Result} = [(r_U - b)/a] \times D$$

r_U = peak response of insulin glargine from the *Sample solution*

b = y-intercept of the calibration curve

a = slope of the calibration curve

D = dilution factor used to prepare the *Sample solution*

Acceptance criteria: 95.0–105.0 USP Insulin Glargine Units/mL

OTHER COMPONENTS

• **Zinc Determination**

Blank: 0.01 N hydrochloric acid

Standard stock solution: 10 µg/mL of zinc in *Blank*, from a commercially available zinc standard solution for atomic absorption

Standard solutions: 0.2, 0.4, and 0.6 µg/mL of zinc from the *Standard stock solution* diluted with *Blank*

Sample solution: Dilute 1 mL of Injection with *Blank* to 100 mL.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Zinc absorption line at 213.9 nm

Flame: Air–acetylene flame of suitable composition (for example, 11 L of air and 2 L of acetylene per min)

Lamp: Suitable radiation source, such as zinc hollow-cathode or electrodeless-discharge-lamp (EDL)

System suitability

Samples: *Blank* and *Standard solutions*

Using the *Standard solutions* and *Blank*, construct a calibration curve by plotting the absorbances of the *Standard solutions* versus their concentrations, and draw the straight line best fitting the three plotted points.

Suitability requirements

Correlation coefficient: NLT 0.999

Analysis

Samples: *Blank*, *Standard solutions*, and *Sample solution*

Determine the concentration, C, in µg/mL of zinc in the *Sample solution* using the calibration curve.

Calculate the quantity of zinc in the portion of Injection taken:

$$\text{Result} = C \times D$$

C = concentration of zinc in the *Sample solution* (µg/mL)

D = dilution factor, 100

Acceptance criteria: 20–40 µg/mL

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **Product-Related Substances**

Mobile phase, System suitability solution, Standard solutions, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual insulin glargine related substance ($\%i_X$) in the portion of Injection taken:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = peak response of the insulin glargine related substance from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Calculate the total percentage of insulin glargine related substances in the portion of Injection taken:

$$\text{Result} = \Sigma\%i_X$$

$\Sigma\%i_X$ = total percentage of insulin glargine related substances from the *Sample solution*

Acceptance criteria

Any individual insulin glargine related substance: NMT 0.5%

Total insulin glargine related substances: NMT 2.0%

Delete the following:

▲• **Limit of High Molecular Weight Proteins**▲ (USP 1-Dec-2022)

Add the following:

▲• **Physicochemical Analytical Procedures for Insulins** ⟨121.1⟩, *Limit of High Molecular Weight Proteins*: Meets the requirements

Acceptance criteria: NMT 0.5%▲ (USP 1-Dec-2022)

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.5–4.5

Change to read:

• **Bacterial Endotoxins Test** ⟨85⟩: ▲Meets the requirements▲ (USP 1-Dec-2022)

• **Sterility Tests** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements

• **Particulate Matter in Injections** ⟨788⟩: Meets the requirements for small-volume injections

• **Injections and Implanted Drug Products** ⟨1⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in the unopened multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protected from sunlight, and avoid freezing.

• **Labeling:** States that it has been prepared with Insulin Glargine produced by methods based on recombinant DNA technology. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Glargine Units/mL.

• **USP Reference Standards** ⟨11⟩

USP Insulin Glargine RS

USP Insulin Glargine for Peak Identification RS

Contains insulin glargine and 0^A-Arg-insulin glargine.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
INSULIN GLARGINE INJECTION	Jennifer Tong Sun Senior Scientist II	BIO2 Biologics Monographs 2 - Proteins

Chromatographic Database Information: Chromatographic Database

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